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A novel tissue for islet transplantation in diabetics

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Abstract

Background—Islet transplantation for diabetes therapy has remained a challenge. None of the currently used transplantation sites have provided satisfactory results as islets seem to require a specific tissue for survival and growth. Since the submandibular gland (SMG) shares physiological and anatomical similarities with the pancreas, we attempted to use this tissue as the transplantation site.

Methods—In Experiment 1, a group of 10 female Syrian Golden hamsters' (SGH) received isolated and purified homologous islets transplanted into their left SMG. In Experiment 2, 15 female SGH received islet transplant into their left SMG as above, except that the recipient hamsters were made diabetic by streptozotocin (STZ) before islet transplantation. In Experiment 3, isolated and purified human islets were transplanted into the SMG of 10 female hamsters

Results—In 8 out of 10 hamsters in Experiment 1 the islets survived and showed the same morphological structure and endocrine cell content, as intrapancreatic islets and presented signs of rapid growth and distribution. Also, as in Experiment 1, well-established islets were present in Experiment 2. Ten of the 15 hamsters pretreated with STZ had blood glucose values between 96 and 125 mg/dl, whereas three hamsters remained hyperglycemic (glucose levels between 194 and 417 mg/dl). Remarkably, the islets in the pancreas of 10 STZ-treated hamsters with functioning SMG islets remained atrophic even after 12 weeks. In two hamsters transplanted islets showed degeneration and remained diabetic until their pancreatic islets regenerated. In Experiment 3, transplanted human islets were completely destroyed.

Conclusions—SMG appears to be the most suitable site for islet transplantation for the treatment of diabetes.

Keywords

Islet; transplant; Syrian hamster; submandibular gland; diabetes; Streptozotocin

INTRODUCTION

Therapy for diabetes, especially of Type 1 diabetes has remained a challenge. The most logical approach for controlling diabetes, namely transplantation of islets either as isolated islets or as pancreas transplantation, did not deliver the expected results. One of the major problems in islet transplantation was the choice of host tissue. The currently favored transplant site, the liver, remains an imperfect treatment. Contrary to experimental results, different transplantation sites or the use of reprogrammed pluripotent stem cells showed similar disappointing results. Induction of islet cell neogenesis by glucagon-like peptide 1 agonists, gastrin, epidermal growth factor and islet neogenesis-associated protein (INGAP) are thought to stimulate neogenesis in rodents, but researchers have yet to find evidence of similar regeneration in humans. Also, other suggested possibilities, such as reprogrammed acinar cells to produce beta cells by injecting them with several transcription factors, or the use of mesenchymal stem cells, that are already being extensively studied for treatment of cardiovascular disease and other conditions, are presently illusive.

Despite numerous recommendations and approaches for successful replacement of nonfunctional islet cells, one fundamentally important physiological aspects of the issue has been largely ignored. Why are islets embedded within the pancreas and not as other endocrine tissues as a compact cell mass anywhere in the body? Also, why are these endocrine elements distributed within the pancreas with tight contact with the surrounding exocrine cells of the pancreas? We do not exactly know the answer, but we realize that their function requires pancreatic tissue where they find the optimal environment for their survival and function. As our numerous previous attempts to maintain islets transplanted into the liver, spleen and subcapsular region of the kidney failed (unpublished results), we were looking for tissues with a structure that resembles the pancreas. Therefore, we chose the submandibular gland and found that this tissue was a unique and ideal place for pancreatic islets to grow and function.

MATERIAL AND METHODS

Animals

Eight-week-old out bred female Syrian Golden hamsters (SGH) of the Eppley colony were used. They were housed in the centralized Comparative Medicine Animal Facilities, an AAALAC International accredited animal facility, in plastic cages on corncob bedding (Bed-O-Cobs, The Anderson Cob Co., Maumee, OH) under standard laboratory conditions (temperature, $21 \pm 2^{\circ}\text{C}$; humidity, $40 \pm 5\%$; light/dark cycle, 12 hr/12 hr; 10x air changes/hr). They were fed a commercial diet (Wayne Lab Blox, Allied Mills, Chicago, IL) and had free access to tap water. The maintenance and humane treatment of the animals followed the guidelines of the UNMC Animal Care and Use Committee.

Islet isolation and culture

Pancreatic islets of eight-week-old female SGH were isolated, purified and cultured as reported [1].

Human islets from a 38-year old male Caucasian healthy multiorgan donor who died of ruptured cerebral aneurysm was kindly provided by the Diabetes Research Institute, University of Miami School of Medicine, Miami, FL. The islets were prepared and purified as reported[2].

Plasma glucose was assayed by Glucose Oxidase method from blood samples taken after an overnight fast. The assays were performed before, 3 days and 4 weeks after streptozotocin treatment and at autopsy. Blood samples were taken from orbital vein of live hamsters and from the right heart at autopsy. Comparison of glucose levels was done by X^2 test.

Experiment 1

Islet transplantation

400 freshly isolated and purified islets from 8-weeks-old female SGH were drawn into a 1-ml Hamilton Syringe modified with a pipette tip attachment. The settled islet pellet was slowly delivered into a 25-cm piece of PE-50 tubing with a 26-gauge needle attached as reported [3]. The tube tip was folded over and spun to form a packed pellet of islets. Under Phenobarbital anesthesia (50 mg/kg body weight), a 1-cm incision was made between the mandibular ridge and the clavicle. Islets were slowly injected into the fully exposed right submandibular gland (SMG) of 10 female SGH. The needle was inserted in the median lower pole of the SMG and pushed slowly and carefully into the upper left pole. While retracting the needle, islets were injected in the needle track. After injection, the SMG was returned to the normal position and the skin was closed by stainless steel surgical clips.

Experiment 2

In the subsequent experiment, a group of female hamsters received streptozotocin (Upjohn, Kalamazoo, MI, 50 mg/kg intraperitoneally) and the glucose levels were determined by retro orbital blood glucose readings. Fifteen hamsters with a reading greater than 200 mg/dl one day after streptozotocin treatment were selected. Three days after streptozotocin treatment, all hamsters received about 750 islets each in their right SMG as described above. They were then euthanized after 12 weeks and their SMG was subjected to a thorough microscopic examination. Glucose levels, using glucose oxidase methods, were assayed before transplantation and at the end of the study. The tissue sections were stained with H&E and processed for immunohistochemistry for the demonstration of the β , α , δ and PP cells, as well as TGF- α and Nestin by a multi-labeling technique developed in our laboratories [4]. Antibodies to insulin, glucagon, somatostatin and PP were obtained from Biogenex Lab Inc. (Fremont, CA), anti TGF- α from Biovision (Mountain View, CA) and anti-Nestin from abcam (Cambridge, MA).

Experiment 3

Using the same technique as in Experiment 2, isolated and purified islets of a 38-year old male was transplanted into the SMG of 10 female 8-week-old hamsters.

RESULTS

Experiment 1

Islets of Langerhans transplanted into the SMG of SGH survived and grew in 8 out of 10 recipient hamsters, where isolated or groups of well-preserved islets were either embedded within the acinar tissue as isolated islets with a well-defined margin (Fig 1), or were in groups close to large or small ducts (Fig. 2), some showing a connection with the ductal wall. The structure of the islet cells, the orthotopic arrangements of individual islet cell types were consistent with that in the pancreas (Fig. 1). About 70% to 80% of the cells reacted with the anti-insulin antibody, about 20% with anti glucagon, 4% with anti somatostatin and only a few with anti-PP, as is also the case in the pancreatic islets. Similar to pancreatic islets some cells immunoreacted with anti-TGF- α and anti-nestin (Fig. 1c) indicating the vitality and maturation of the cells.. Although the boundary between the glandular tissue and islets were well defined, as in the pancreas, isolated or small groups of beta cells were present in the vicinity of the islets attached to the exocrine cells in each islet (Fig. 1c). Many of the islets showed good vascularization and some contained congested blood vessels (Figs. 1a). The number of islets per each 10mm section of the gland varied between 2 and 10 but more islets was discernable in serially cut tissues along the route of the injection track. They were either clustered together or distributed in different areas of the gland as small or large clusters (Fig. 2). Some of the islets were large (800-1000 μ), whereas others were composed of about 200-500 cells. Remarkably, in three cases, the islet clusters presented branching mimicking the pattern of invasive tumor (Fig. 2a). Histologically, the acinar tissue surrounding the islets did not show any abnormalities. The failure to find islets in two of the 10 hamsters could be related to the insufficient number of transplanted islets, some of which may have drained out from the needle track.

Experiment 2

All but two hamsters receiving islet transplant showed a well-established islet cluster in their SMG. Although the toxic effects of streptozotocin usually subside after three days [7], in two hamsters only a few degenerated islets, identical to the pancreatic islets of streptozotocin treated hamster, but no intact islets were found. In 10 hamsters with preserved islets in the SMG, the pancreatic islets of STZ-treated hamsters remained atrophic even after 12 weeks. In these hamsters blood glucose values, **which was assayed at the end of the study** varied between 96 and 125 mg/dl, whereas the remaining three hamsters remained hyperglycemic (blood glucose levels between 194 and 417 mg/dl), which was significantly higher than those in 10 hamsters. The two hamsters with damaged islets in their SMG recovered from their diabetes (glucose levels 130 and 140 mg/dl at autopsy) and sowed near normal pancreatic islets with scattered balloon cells in a few islets.

Experiment 3

In all 10 hamsters transplanted, human islets showed degeneration and formation of foreign body granuloma (Fig. 3). These hamsters recovered from diabetes after 14 days and showed normal islets in their pancreas at autopsy at 12 week. Their final blood glucose levels ranged between 95 and 130 mg/dl.

DISCUSSION

Several attempts to cure diabetes by islet transplantation into a variety of tissues have failed as islets show resistance to survive and function properly in selected sites. Also, transplantation of part or the whole pancreas did not provide the expected results. In such attempts, the physiological and biological patterns of the Langerhans islets have not been taken into consideration. It appears that certain components of the exocrine pancreas provide a suitable niche for the islets. The identity of the responsible specific factor is unknown but the presence of acinar and ductal cells appears to be fundamental. In such a case, any tissue that contains acinar and ductal elements could be a second home for the islets. With this in mind, we decided to test the SMG [5, 6] as the suitable territory for homologous islets. The results of this study demonstrate that islets could be transplanted successfully in the SMG, where they survive, retain their secretory capacity and cure induced diabetes in about 70% of the cases. The presence of acinar and ductal cells, along with the presence of growth factors in this gland, seem to be responsible for the rapid expansion of transplanted islets. Although we did not examine the rate of DNA synthesis and replication in the transplanted isles, the remarkable expansion of islets into the surrounding exocrine tissue and the presence of extra insular islet cells in the vicinity of islets undeniably point to the replication and renewal ability. The presence of many intrainsular congested blood vessels indicates that they form their own blood vessels in this tissue, which is important for delivering their hormones into the blood circulation. As in the intrapancreatic islets, these islets contained all four endocrine cell types in typical orthotopic arrangements and expressed TGF- α and nestin, implying that these islets have accepted the SMG as a home. Hence, it appears that this tissue could be used successfully for the therapy of diabetes. In this context, it must be pointed out that our numerous previous attempts to maintain islets transplanted into the liver, spleen and subcapsular region of the kidney failed (unpublished results) indicating that the islets require a unique environment for their growth and function. The results obtained from two hamsters whose transplanted islets showed signs of degeneration and high glucose levels highlight a direct correlation between the integrity of islets in the SMG and the blood glucose levels. Moreover, these two hamsters recovered from diabetes by virtue of the islet cell regeneration, which occurs post streptozotocin treatment [7].

STZ-induced diabetes differs in many aspects from human type 2 diabetes in that islets destroyed by STZ recover within 12 weeks (6). Hence, the normalization of the glucose level at 12 weeks could argue against the functional activity of the transplanted islets for diabetes recovery. However, the presence of atrophic islets in all STZ-treated hamsters at 12 weeks ultimately negate this possibility and indicate that the improvement of glucose metabolism was not achieved by intrapancreatic insulin. This view was substantiated by the two cases, where transplanted islets were degenerated and in whom islet damage by streptozotocin was repaired. Also contrary to the other hamsters with functioning SMG islets and atrophic pancreatic islets, these two hamsters did not show pancreatic islet atrophy. This observation along with the sustained atrophy of islets in the STZ-group and the lack of regenerative signs was consistent with our previous study showing that extra-insular insulin treatment inhibits the regeneration islets destroyed by STZ [7]. Consequently, insulin seems to control the secretion and the vitality of the β -cells. Since glucose levels were studied only

at the start and the end of the experiment, the data do not provide information on transplant/graft function. Further studies are needed for clarification.

Whether or not the functional integrity of transplanted islets in SMG is a species related phenomenon presently remains unanswered. It could be argued that the ease in islet survival within the SMG could be related to the immunological competency of the hamster tissue due to inbreeding of hamsters in a closed colony. This argument is refuted by the fact that islets transplanted into the renal subcapsular region, spleen, liver and cheek pouch elicited immunological reaction and rejection, as did the human islets in SMG, which was rejected by heavy inflammation and destruction indicating that the SMG provides a suitable home for islets. Although the scientific reasons for the proper environment of SMG are yet obscure we hypothesize that based on the established tight connection between islets and ductal cells, from which islets are produced, islets require ductal/ductular tissue for survival and can accept any glandular tissue as home.

Abbreviations

SGH	Syrian Golden Hamster
SMG	Submandibular glands
STZ	Streptozotocin

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Figure 1.

Transplanted islets within the submandibular tissue of a SGH. **a)** Two well-preserved islets, immunoreactive with anti insulin (*blue*) with the glandular tissue. Although the boundary of the islets is well delineated, a few cells appear to extend beyond the boundary into exocrine tissue (*black arrow*). Large and congested blood vessels were seen in many islets (*white arrows*). X65, anti-insulin antibody) and H&E. **b)** A single, well-demarcated islet in the SMG containing insulin (*brown*) and glucagon cells (*blue*). X 65, double staining with anti-insulin and anti-glucagon) and H&E. **c)** An islet within the SMG tissue composed primarily of insulin (*brown*) and glucagon (*blue*). Note the presence of a single insulin cell in the exocrine tissue adjacent to the islet (*arrow*). X65, double staining with anti-insulin and anti-glucagon) and H&E. **d)** Expression of glucagon (*blue*) and TGF- α (*red*) in a large islet. The central portion of the islet contains β -cells. X75, double staining with anti-glucagon and antiTGF- α .

Figure 2.

a) A large number of transplanted islets in the SMG of a female hamster composed primarily of insulin cells (*brown*) and glucagon cells (*blue*). Remarkable arrangement of the islets in columns and arrow forms in a pattern reminiscent of invasive growth. A large cyst is seen (*upper right corner*). X10, double staining with anti-insulin and anti-glucagon). The staining of the exocrine tissue was omitted for better visualization of the islet pattern. **b)** Several groups of islets within the SMG tissue. There are small groups of insulin cells (*brown*) scattered with the exocrine tissue (*arrows*), a pattern that also exists in the pancreas

(so-called “extra insular islet cells”) x50, anti-insulin and H&E staining. **c)** Granulation tissue in the SMG of a female hamster after human islet transplantation. X65. H&E staining.

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Figure 3. Degeneration of islet cells in SMG of hamsters who was treated with streptozotocin 3 days earlier. Marked swelling and ballooning of islet cells surrounded by inflammatory cells.